Fisheries and Environment
Forestry Service

EFFECT OF EXOGENOUS GROWTH SUBSTANCES ON FLOWERING IN GRAFTED CLONES OF **LARIX**

by J. Peter Hall

NEWFOUNDLAND FOREST RESEARCH CENTRE
ST. JOHN'S, NEWFOUNDLAND
INFORMATION REPORT N-X-151

NOVEMBER 1977

Fisheries and Environment

Forestry Service

EFFECT OF EXOGENOUS GROWTH SUBSTANCES ON FLOWERING IN GRAFTED CLONES OF *LARIX*

by J. Peter Hall

NEWFOUNDLAND FOREST RESEARCH CENTRE
ST. JOHN'S, NEWFOUNDLAND
INFORMATION REPORT N-X-151

NOVEMBER 1977

TABLE OF CONTENTS

	Page
INTRODUCTION	1
METHODS	2
RESULTS	3
DISCUSSION	6
CONCLUSIONS	9
LITERATURE CITED	10
APPENDIX I	11
APPENDIX II	12

ABSTRACT

Conifer breeding research would be greatly facilitated if early and consistent flowering could be ensured. Physiological methods of flower stimulation using gibberellins and auxins have shown promise in preliminary studies on conifers. This study was designed to stimulate flowering and alter sex ratios in L. decidua and L. kaempferi by application of exogenous GA_3, NAA, TIBA and 6BA. Growth substances were applied at concentrations of 0, 1, 10, 100 and 1000 ppm in April and May.

Levels of female flowering declined and levels of male flowering increased followed application of the auxin NAA on L. kaempferi. The anti-auxin, TIBA, had the opposite effects. The data from all treatments on L. decidua and with GA_3 and 6BA on L. kaempferi were inconclusive.

Recommendations are made for future studies on flower stimulation in Larix.

RÉSUMÉ

La recherche sur l'hybridation de coniferes serait grandement facilitée si une floraison précoce et consistante était assurée. Des méthodes physiologiques de stimulation des fleurs utilisant des gibberellines et auxines semblent prometteuses à la suite d'études préliminaires sur les conifères. Cette étude était désigneé pour stimuler la floraison et d'altérer la proportion des fleurs mâles et femelles chez L. decidua et L. kaempferi par l'application exogéne de GA_3, NAA, TIBA et 6BA. Les substances de croissance étaient appliquées à des concentrations de 0, 1, 10, 100 et 1000 ppm, en avril et en mai.

Les taux de fleurs femelles décroissent et ceux de fleurs mâles augmentent à la suite de l'application de l'auxine NAA sur L. kaempferi. L'anti-auxine TIBA avait des effets contraires. Les données de tous les traitements sur L. decidua et des traitements de GA_3 et 6BA sur L. kaempferi n'étaient pas concluantes.

Des recommandations sont faites pour des études futures sur la stimulation des fleurs chez les Larix.

EFFECT OF EXOGENOUS GROWTH SUBSTANCES ON FLOWERING IN GRAFTED CLONES OF *LARIX*

by

J. Peter Hall

INTRODUCTION

Some of the problems encountered in breeding programs with conifers are the long juvenile period and the variation in levels of flowering in different years. These problems cause delays and interruptions in research programs, therefore, if early and consistent flowering could be assured it would result in considerable increase in the efficiency of tree breeding research. In addition, it would be advantageous to be able to alter the ratio of male to female flowers or to induce male or female sterility.

Flowering levels of conifers have been altered by cultural methods such as girdling of stems, pruning of branches or roots, manipulation of branch angle, water stress and fertilization but results have varied with season, year and species (Audus, 1972).

In recent years physiological methods for promoting flowering have been intensively studied. One technique is the application of exogenous growth substances which has been used to manipulate flowering in herbaceous angiosperms (Audus 1972. Waering and Phillips 1970). Stimulation of flowering in conifers with gibberellins was reported by Kato *et al*. (1958) for *Cryptomeria japonica* and by Hashizume (1967) for *Larix kaempferi*. Dunberg (1974), reported that clones of *Picea abies* which consistently flower, had relatively higher levels of gibberellin-like substances at the time of supposed flower bud initiation than clones which produce few or no flowers.

A study was established in 1975 with the objectives of stimulating flowering and altering sex ratios by the application of growth substances as part of an investigation on seed production in *Larix* (Hall 1976). It was done at the UK Forestry Commission Tree Nursery at Newton in Morayshire Scotland (Lat. 57° 39'N Long. 3° 21'W Elev. 24 m). Since the flowering study was exploratory, the range of tree material, numbers of growth substances and their concentration was of necessity restricted.

METHODS

Few experiments involving the application of growth substances to Larix have been reported in the literature and the information was limited regarding (i) growth substances which may affect flowering, (ii) concentration of substances, (iii) the method of application or (iv) the time of application.

(i) Growth Substances. Gibberellic Acid (GA_3) was used in this study because it has been shown to promote flowering in many angiosperms and in conifers of the Cupressaceae. An auxin-1 (Naphthylacetic acid (NAA)) was also used since Hashizume (1967) successfully promoted flowering in L. kaempferi with this substance. Because the balance of growth substances within the plant appears to be important in the control of growth processes, an anti-auxin (2,3,5-Tri-iodo acid (TIBA)) was applied to the trees. A cytokinin (6-benzyl-aminopurine (6BA)), was chosen to test effects on flowering and sex expression.

(ii) Concentration of Growth Substances. There is very little information from the literature concerning the optimal concentration of growth substances for application on larch. Hashizume (1967) reported stimulation of flowering in L. kaempferi using GA_3 and NAA at 1000 and 4000 ppm. In this study four concentrations were used; 1, 10, 100 and 1000 ppm plus a water control. Growth substances were dissolved in distilled water at a concentration of 1000 ppm and then diluted.

(iii) Method of Application. Application of growth substances by foliar sprays have been used for many plants, however, since there was very little foliage on the test clones in April, the method described below was used. Specimen bottles (capacity 25 cc) were tied to the underside of the branch and a wick enclosed in rubber tubing was drawn up through a small hole drilled vertically through the branch. The hole was sealed with 'Arbex', a commercial sealing compound. The purpose of the rubber tubing was to reduce evaporation of the solution[1]. The bottles, with rubber tubing were then tied to the branches.

(iv) Time of Application. In order to be effective, growth substances must be applied before the floral structure has been irreversibly determined. Longman et al. (1965) found that flower initiation could be arrested or promoted in L. kaempferi growing in England (approx. 5° Lat. south of the study area) by altering branch angle during April to mid-May. Since times of flushing are later in northeast Scotland the growth substances were applied during the last week in April and May.

---

[1]Additional sample bottles with wicks and rubber tubing were tied to the branches to assess the amount of evaporation.

(v) Study Design. The study was divided into two parts in order to evaluate the effects of the growth substances in terms of possible variation among clones and to test different dates of application. In Part A the four growth substances were applied on one clone each of L. kaempferi (J130) and L. decidua (E436) and replicated three times. Each graft was treated with one growth substance applied at 5 concentrations (0, 1, 10, 100, 1000 ppm) and each was applied to a different branch in the lower part of the crown. Application was carried out during the last week of April.

In Part B, Gibberellin (GA_3) was applied to four different clones of L. kaempferi (J312, 242, 250, 441) and two of L. decidua (E140, 332) on 23 April and again on 23 May. Two of the L. kaempferi (J312, 242) clones and both L. decidua clones received GA_3 at 5 concentrations (as in Part A). The remaining two clones of L. kaempferi (J250, 441) were treated with GA_3 only at 0 and 1000 ppm.

(vi) Collection of Data. After two months the bottles were removed and the amount of liquid absorbed by the branches was recorded. The effects of the treatments were assessed in March 1976 by counting the total numbers of flowers per metre of branch for each treatment. Approximately five metres of branch were sampled (randomly) for each replicate of each treatment.

RESULTS

The number of flowers counted and length of branches measured are shown in Appendices I and II.

The amount of liquid absorbed (Table 1) by the branches in Part A ranged from 1.2 to 22.5 cc; most branches absorbed between 5 and 15 cc. Branches in clone J130 generally absorbed more liquid than those in clone E436. In Part B branches generally absorbed more of the liquid than those in Part A. The sample bottles remained full throughout the period indicating that no evaporation occurred.

Table 2 shows the number of flowers and the ratio of male to female flowers for both clones treated with the four growth substances at five concentrations. In clone J130 three branches were broken off by windstorms before the data from them could be obtained and in both clones some bottles containing growth substances remained full throughout the study period. These data were, therefore, not included in the results.

(i) Part A.

In clone J130 there were generally fewer flowers of both sexes with increased concentrations of GA_3 (Table 2). Application of NAA resulted in more male flowers, but when TIBA was applied the

Table 1.- Volume of liquid (cc) absorbed by the branches in each treatment (mean of three replications).

(i) Part A

Clone	Growth substance	Concentration of growth substance (ppm) 0	1	10	100	1000
		Amount of liquid absorbed by branch				
J130*	GA_3	2.5	0.0	5.4	6.2	5.0
	NAA	5.0	15.0	5.0	2.5	17.5
	TIBA	11.7	3.1	3.8	6.2	10.0
	6BA	3.8	10.0	4.4	3.8	10.0
E436	GA_3	5.0	22.5	2.5	1.2	5.0
	NAA	7.5	1.9	5.0	6.2	9.2
	TIBA	5.0	2.5	5.0	0.0	6.9
	6BA	0.0	3.8	0.0	3.8	0.0

(ii) Part B

Clone	Date					
J312	23 April	7.5	2.5	2.5	10.0	5.0
	23 May	17.5	15.0	0.0	25.0	25.0
J242	23 April	0.0	7.5	17.5	0.0	1.2
	23 May	10.0	15.0	22.5	25.0	20.0
J250	23 April	25.0	-	-	-	20.0
	23 May	25.0	-	-	-	12.5
J441	23 April	20.0	-	-	-	17.5
	23 May	15.0	-	-	-	17.5
E140	23 April	2.5	2.5	0.0	2.5	7.5
	23 May	12.5	7.5	7.5	25.0	20.0
E332	23 April	12.5	2.5	0.0	0.0	5.0
	23 May	15.0	15.0	15.0	10.0	20.0

*J = L. kaempferi, E = L. decidua

Table 2.- Number of flowers and ratio of male (M) to female (F) flowers on L. kaempferi (J130) and L. decidua (E436) treated with 4 growth substances at 4 concentrations (Part A).

(i) Numbers of flowers per metre of branch.

Clone	Growth substance	Concentration in parts per million: 0		1		10		100		1000	
		Numbers of flowers per metre of branch: M	F	M	F	M	F	M	F	M	F
J130	GA_3	60.6	4.8	-	-	49.7	1.6	59.1	0.2	23.5	0.3
	NAA	65.2	2.2	62.5	2.0	78.6	1.9	44.1	1.4	85.4	0.4
	TIBA	58.2	2.3	47.2	5.1	53.9	5.4	55.0	3.0	51.9	9.1
	6BA	63.6	1.5	55.4	2.6	65.0	0.7	48.4	2.9	45.8	2.9
E436	GA_3	36.2	0.0	38.3	0.0	31.5	0.0	34.7	0.0	29.7	0.2
	NAA	39.9	0.0	38.4	0.0	35.4	0.4	33.4	0.0	27.6	0.0
	TIBA	31.1	0.4	36.9	0.4	38.2	0.2	-	-	39.0	0.4
	6BA	-	-	37.0	0.2	-	-	40.0	0.6	-	-

(ii) Ratio of male to female flowers.

J130	GA_3	12.5	-	31.1	311.0	85.0
	NAA	30.2	30.9	41.2	32.3	222.5
	TIBA	25.8	9.2	10.0	18.4	5.7
	6BA	41.6	21.4	97.1	16.6	15.9
E436	GA_3	-	-	-	-	169.0
	NAA	-	-	94.0	-	-
	TIBA	78.5	102.5	214.7	-	93.0
	6BA	-	189.0	-	62.8	-

numbers of male flowers decreased. Application of TIBA, however, resulted in increased numbers of female flowers. Application of 6BA resulted in fewer male and more female flowers except at concentrations of 10 ppm where these trends were reversed.

Concerning the ratio of male to female flowers, application of GA_3 and NAA increased the ratio. Application of TIBA and 6BA resulted in decreased ratios, except when 6BA was applied at a concentration of 10 ppm.

In clone E436 the levels of flowering seemed unaffected by application of GA_3 and 6BA. The numbers of male flowers tended to decrease when NAA was applied and increase when TIBA was applied. Few female flowers were formed and no effect of treatment was apparent.

(ii) Part B.

Table 3 shows the numbers of flowers on clones treated with GA_3 on the two dates (Part B). In clone J312 the numbers of female flowers were increased over the control on both dates, the greatest numbers of flowers occurred at a concentration of 10 ppm. The level of flowering in clone J242 was unaffected by the treatments. In clone J250 treated on 23 April and in clone J441 on both dates female flowering was reduced. Clone J250 treated on 23 May showed an increase in the level of flowering. The numbers of male flowers in all clones appeared generally unaffected by the treatments.

The L. decidua clones had very few female flowers and the treatments had no effect. The numbers of male flowers on treated branches were fewer than on the controls only at a concentration of 10 ppm.

DISCUSSION

Because there were so few female flowers in the L. decidua clones it was impossible to conclude whether or not treatments had a differential effect on either flowering or sex expression.

In the L. kaempferi clones the application of GA_3 appears to suppress rather than promote flowering. This is in contrast to Hashizume (1967) who reported stimulation of flowering using GA_3 at concentrations of 1000 and 4000 ppm. Following the establishment of the study reported here, Pharis (1976) reported that flowering in grafted seedlings of Pseudotsuga menziesii was not stimulated by GA_3 but was stimulated by a combination of GA_4, GA_7 and GA_9. These latter substances are non-polar and less oxidized gibberellins than GA_3.

Table 3.- Number of flowers in clones of L. kaempferi and L. decidua after treatment with GA_3 (Part B).

		Concentration in parts per million									
		0		1		10		100		1000	
		Number of flowers per metre of branch									
Clone	Date treated	M	F	M	F	M	F	M	F	M	F
J312	23 April	46.1	1.5	56.5	2.7	37.8	4.4	45.5	2.7	39.2	2.6
	23 May	46.8	0.0	35.7	1.4	-	-	39.0	2.6	49.9	1.5
J242	23 April	-	-	49.2	0.2	47.8	0.6	-	-	58.4	0.0
	23 May	64.1	0.0	41.6	0.0	61.8	0.2	57.0	0.2	86.1	0.0
J250	23 April	49.9	18.9	-	-	-	-	-	-	54.0	10.5
	23 May	52.5	2.6	-	-	-	-	-	-	54.9	8.5
J441	23 April	62.6	4.4	-	-	-	-	-	-	64.1	3.5
	23 May	42.2	9.7	-	-	-	-	-	-	51.1	4.2
E140	23 April	26.8	1.0	30.3	1.1	-	-	26.7	0.4	26.3	0.1
	23 May	36.2	0.2	19.2	0.9	42.8	0.4	35.7	1.4	41.6	0.0
E332	23 April	34.1	0.0	18.7	0.6	-	-	-	-	33.1	0.0
	23 May	41.3	0.0	42.8	0.6	35.1	1.0	43.2	0.8	23.6	1.0

Application of NAA decreases female flowering at all concentrations. Male flowering was decreased at concentrations of 1 and 100 ppm and increased at other concentrations. These results contrast with those of Hashizume (1967) who obtained increased female flowering with NAA on L. kaempferi. Application of the anti-auxin TIBA increases female flowering and decreases male flowering, i.e. the opposite effect to NAA. These results indicate that sex expression in Larix may be altered by the manipulation of auxin levels.

The cytokinin, 6BA has the same effect as the anti-auxin (except at concentration of 10 ppm) and it is possible that it acts in the same fashion as the anti-auxin on levels of flowering and sex expression. It is possible that the anomalous result obtained with NAA at 100 ppm (low level of male flowering) is attributable to the small amount of liquid absorbed by the branch compared to absorption at other concentrations.

CONCLUSIONS

The application of four exogenous growth substances to grafted clones of L. decidua and L. kaempferi generally resulted in decreased levels of male flowering. In L. kaempferi application of GA_3 and NAA suppressed female flowering and the application of TIBA and 6BA stimulated it. The low levels of female flowering in L. decidua made interpretation of, and conclusions from, the results impossible.

The method of application of the growth substances, with bottles and wicks into the branch as indicated by anomalies in the results was not entirely successful.

Studies of the relationship between flowering-sex expression and exogenous growth substances should include other gibberellins, probably GA_4, GA_7 and GA_9 and different techniques for the applications of the treatments.

LITERATURE CITED

Audus, L.J. 1972. Plant growth substances, Vol. 1. Chemistry and Physiology, Leonard Hill, London, 533 pp.

Dunberg, A. 1974. Occurrence of gibberellin - like substance in Norway Spruce (Picea abies (L) Karst) and their possible relation to growth and flowering. Studia Forestalia Suecica Nr. 111, 62 pp.

Hall, J. Peter. 1976. Yield of seed and hybridization in the genus Larix. Miller. Ph.D Thesis Univ. of Aberdeen, Aberdeen, Scotland. 215 pp.

Hashizume, H. 1967. Experimental induction of female flowers in young Japanese larch (Larix leptolepis). J. Jap. For. Soc. 49: 405-408.

Longman, K.A., T.A.A. Nasr and P.F. Waering. 1965. Gravimorphism in trees 4. The effect of gravity on flowering. Annals of Botany Vol. 29: 459-473.

Kato, Y.N., I. Miyake and H. Ishikawa, 1958. Initiation of flower bud by gibberellin in Cryptomeria japonica. J. Jap. For. Soc. 49:405-408.

Pharis, R.P. 1976. Promotion of flowering in conifers by gibberellins For. Chron. Vol. 51: 244-248.

Waering, P.F. and I.D.J. Phillips. 1970. The control of growth and differentiation in plants. Pergamon Press, Oxford. 303 pp.

APPENDIX I

Numbers of flowers and length of branches measured Part A

Clone	Growth substance	Concentrations of growth substance (ppm) 0		1		10		100		1000	
		Numbers of male and female flowers (total)									
		M	F	M	F	M	F	M	F	M	F
J130	GA_3	625	50	-	-	715	23	311	1	85	1
	NAA	1057	35	680	22	825	20	226	7	445	2
	TIBA	955	37	488	53	289	29	884	48	584	103
	6BA	665	16	578	27	680	7	781	47	477	30
E436	GA_3	209	0	220	0	188	0	192	0	169	1
	NAA	435	0	423	0	376	4	364	0	316	0
	TIBA	157	2	410	4	644	3	-	-	465	5
	6BA	-	-	189	1	171	0	628	10	157	0

		Length of branch measured (metres)				
J130	GA_3	10.31	-	14.40	5.26	3.61
	NAA	16.20	10.88	10.49	5.13	5.21
	TIBA	16.40	10.34	5.36	16.08	11.33
	6BA	10.46	10.44	10.46	16.13	10.42
E436	GA_3	5.77	5.74	5.97	5.54	5.69
	NAA	10.90	11.02	10.62	10.90	11.45
	TIBA	5.05	11.10	16.83	-	11.91
	6BA	-	5.11	5.13	15.68	5.43

Note: Appendix includes only data where some liquid was absorbed by the branch (Page 3).

APPENDIX II

Numbers of flowers and length of branches measured - Part B.

Clone	Date of treatment	Concentration of growth substance (ppm) 0		1		10		100		1000	
		Numbers of male and female flowers (total)									
		M	F	M	F	M	F	M	F	M	F
J312	23 April	240	8	290	14	-	-	235	14	199	13
	23 May	246	0	185	7	259	5	193	13	275	8
J242	23 April	-	-	251	5	254	3	-	-	340	0
	23 May	332	0	219	0	347	1	295	1	430	0
J250	23 April	274	104	-	-	-	-	-	-	309	60
	23 May	264	13	-	-	-	-	-	-	297	46
J441	23 April	324	23	-	-	-	-	-	-	352	19
	23 May	222	51	-	-	-	-	-	-	269	22
E140	23 April	136	5	164	6	-	-	135	2	133	5
	23 May	181	1	102	5	121	8	151	1	130	4
E332	23 April	173	0	95	3	-	-	-	-	170	0
	23 May	210	0	245	2	183	5	226	4	120	5
		Length of branch measured (metres)									
J312	23 April	5.21		5.13		-		5.16		5.08	
	23 May	5.26		5.18		5.28		5.08		5.51	
J242	23 April	-		5.10		5.31		-		5.82	
	23 May	5.18		5.26		5.61		5.18		5.03	
J250	23 April	5.49		-		-		-		5.72	
	23 May	5.03		-		-		-		5.41	
J441	23 April	5.18		-		-		-		5.49	
	23 May	5.26		-		-		-		5.26	
E140	23 April	5.08		5.41		-		5.05		5.05	
	23 May	5.00		5.31		5.08		5.16		5.23	
E332	23 April	5.08		5.08		-		-		5.13	
	23 May	5.08		5.72		5.21		5.23		5.08	